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METHODS OF SEARCH FOR ANTIBIOTICS POSSESSING ANTIVIRUS ACTIVITY

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One of the most important and timely problems facing investigators occupied with seeking new antibiotics is the search for antibiotics for therapy of virus diseases, particularly influenza, the most widespread of them.

The prospect of finding antibiotics formed by microorganisms and possessing antivirus properties is indicated by the discovery in recent years of a quite large number of antibiotic substances which check the development or which even destroy various viruses.

Beginning with 1953 our investigations looking for new antibiotics of antivirus activity led to the search for and development of methods suitable for the selection of producers of such antibiotics. We can divide these investigations into several phases. In the first period we worked with the virus of tobacco mosaic which is frequently used for the study of activity of antivirus preparations. We used a method of selection permitting us to study the antivirus activity of cultures of actinomycetes on agar media. At the surface of the agar, thickly overgrown with actinomycete mycelia, 3 agar blocks 9 mm in diameter were excised with a corkscrew which had been flamed. Agar blocks of 3-4 mm thickness were placed along the upper surfaces of datura leaves (Datura stramonium, D. alba, or D. bernhardii) or of tobacco leaves (Nicotiana glutinosa). As is well known, the virus of tobacco mosaic evokes only local spotty necroses on the leaves of these plants; this permits a quantitative account of the intensity of the infection. Twenty to 30 minutes before spreading the agar blocks, the leaves were infected with the virus of tabacco mosaic. After the moistened surface dried, the 3 agar blocks, with actinomycetes growing on them, were spread on one half of the leaf at some distance from one another, while on the other half were spread 3 sterile agar blocks of the same size and composition as the nutritive medium on which the actinomycetes were growing. The leaves with the adherent blocks were kept in a moist room at a temperature of 25-28°C for 4 days. Then the spotty necroses were counted under the blocks and on the free surface of the leaf.

The results obtained are presented in the table below.

Twenty-eight of the 450 strains (6.1%) checked the development of spotty necroses on the datura or Nicotiana glutinosa leaves. Only the strains which gave identical results with 2 or 3 repetitions of the experiments are included in this group. For 13 of these streins the inhibition of the development of necroses was observed only under the agar blocks, while for 15 of them the involvement was absent not only under the blocks but also for some distance from them. Apparently in the first case the active compound formed by the fungus did not diffuse into the tissues of the leaf, whereas in the second case a more or less clear-cut diffusion of the antibiotic substance occurred. From

the cultures of one of these strains chemists of the Institute of the Search for New Antibiotics AMN SSSR isolated an antibiotic substance called antivirubin, which suppresses the growth of Staphylococcus aureus and which possesses a strong antivirus action.

Thus tobacco mosaic disease can be used for the primary selection of producers of antivirus antibiotics. These first observations indicate a wide distribution of substances which neutralize the tobacco mosaic virus among the products of metabolism of actinomycetes.

INFLUENCE OF ACTINOMYCETES ON THE DEVELOPMENT OF AFFLICTIONS CAUSED BY THE VIRUS OF TOBACCO MOSAIC

No of group	Character of Affliction	Number of strains
1	Number of spotty necroses under the blocks and on the free surface of the leaf approximately the same	384
2	Number of spotty necroses under the blocks much less than on the free surface of the leaf or else completely absent	13
3	Necroses absent not only under the blocks but also for some distance around them	15
4	Necroses under the blocks more or strongly manifest than on the remaining part of the leaf	8
5	Results indistinct	30

In the next phase of our investigations we took up the study of viricidal substances of the culture fluids.

The culture fluid was mixed in various quantities with suspensions of the virus of tobacco mosaic and at various intervals of time, usually 24 hours after its preparation, the suspension was applied to the surface of datura or Nicotiana glutinosa leaves. In 3-4 days the spotty necroses formed were counted. In view of the difficulties connected with obtaining tobacco or datura leaves in the winter and also in order to work with animal viruses, we proceeded to the influenza virus in 1954. The method adopted by us culminated in the determination of neutralizing activity of culture fluids on the influenza virus in vitro. We worked with the influenza A_1 virus. Suspension of the virus in a dilution of 1:1,000 was mixed with an equal quantity of culture fluid or antibiotic solution and carefully mixed; the suspension was maintained for 3 hours at room temperature. The initial pH of the culture fluid or solution of antibiotics was brought to 7.3 with the greatest possible accuracy. In 3 hours the suspension was diluted again by 5 times. Thus the final dilution of the suspension of virus was 1:10,000. Each suspension served for the intranasal infection of 5 mice. As the findings showed, the experimental mice received more than 100 fatal doses of the virus.

The infected animals, experimental as well as control, were under observation for 10 days after which the mice remaining alive were killed and the degree of involvement of the lungs was determined macroscopically in them. The control mice, infected by suspension of 1:10,000

concentration, died on the fourth to sixth day after the onset of the experiment.

The question arises as to how well grounded the search is for antibiotic substances for the therapy of virus diseases according to the neutralizing action of the preparations in vitro. Considering the recently published work on cycles of development of bacteriophage and viruses of animals, we believe that such a method is attainable. According to the latter data viruses within the cells develop in the form of special vegetative viruses, devoid of infectious properties, while only mature infectious particles leave the cells and involve the other, healthy cells; therefore it is quite obvious that it is easier to affect the extracellular phase of development of the virus with chemotherapeutic substances than the intracellular phase. This is the most vulnerable link in the development cycle of the virus. There is some risk presented by the fact that by this method basically antibiotics will be selected which combine with the protein membrane of the virus, and, accordingly, with proteins in general. Actually, 8 of the antivirus antibiotics obtained in our institute neutralized the blood serum proteins to a greater or lesser degree.

According to the above method 212 culture fluids taken from the microbiology laboratory of the institute and 192 antibiotics were checked; and most of the antibiotics were obtained at the Institute of the Search for New Antibiotics.

Of the 192 antibiotic preparations 30 (15.6%) neutralized influenza virus in vitro. Twelve of these preparations possessed clear therapeutic actions in the treatment of mice infected intranasally by small quantities of fatal doses of influenza virus, by the method of subcutaneous injections, or by inhalations. Accordingly, among the antibacterial antibiotics formed by actinomycetes, there are encountered quite a number of agents which neutralize the influenza virus and which possess chemotherapeutic effects in virus infection of mice.

Interesting results were obtained also in the investigation of the 212 culture fluids, most of which were preliminarily selected according to their action on the tobacco mosaic virus; 156 of them (73.6%) did not produce an unfavorable effect on the influenza virus. Under these conditions 56 (26.4%) partially or completely neutralized the influenza virus. The chemists of the Institute of the Search for New Antibiotics obtained 13 antibiotic agents from 13 culture fluids which acted on the virus of influenza. Eleven of the 13 antibiotics obtained (85%) neutralized the influenza virus in vitro. These observations show that a considerable number of actinomycetes excrete agents which neutralize the virus of influenza in vitro and point to the broad prospect of seeking out antivirus agents among the products of activity of the actinomycetes.

In conclusion we should like to make the following comments. Two of the antibiotics obtained at the institute which neutralize influenza virus in vitro were inactive with respect to the poliomyelitis virus (according to the data of professor M. P. Chumakov) and to the Taylor virus. Antivirubin, which neutralizes the influenza smallpox, and tobacco mosaic viruses, did not act on A. aerogenes bacteriophage. In comparing the results obtained with the influenza virus to 122 culture fluids with the analogous results for the tobacco mosaic virus of R. S. Ruchkina, coincident results were obtained for the 2 viruses in 80% of the cases. In the literature on the subject many examples of

the selctive action of antibiotics on various viruses can be found. Thus fumagillin acts only on bacterial viruses but does not affect animal viruses. Phagolessin neutralized only about 30 bacteriophages out of 50 and also the viruses of smallpox vaccine and poliomyelitis, but did not act on other viruses. More precisely, it may be said that all antivirus antibiotics which have been isolated in recent times have their specific antivirus spectrum. As examples we have: erlichin, achroviromycin, netropsin, cardicidin, abikoviromycin, viscosin, leucomycin, grasseriomycin, and many others.

Thus viruses in this respect are apparently no different from bacteria. Therefore in searching for antivirus antibiotics not just one kind of virus should be used but a definite set of them, which should contain the basic representatives of this large group. Study should be conducted in vitro, in vivo, and in tissue culture experiments.

Conclusions

1. Actinomycete-secreted antagonistic substances which neutralize the influenza or the tobacco mosaic virus in vitro are frequently encountered under natural conditions.

2. Among 30 antibiotics obtained which neutralize the influenza virus in vitro, 12 possessed more or less clearly manifest chemotherapeutic effects on mice infected with small quantities of fatal doses of influenza virus. However the majority of preparations obtained were either very toxic or poorly effective.

3. Antivirus antibiotics as well as antibacterial preparations possess a definite spectrum of action. Therefore a rational choice of antivirus antibiotics should be made not only with respect to one species of virus, but also with respect to the basic representatives of this large group.

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